THE UNIVERSITY OF KANSAS
SCIENCE BULLETIN

VOL. XVII.] SEPTEMBER, 1927. [No. 5.

Some Studies on the Structure and Behavior During Germination of *Gymnocladus canadensis* Lam.

W. H. HORR, Department of Botany.

INTRODUCTION.

IN THIS paper an attempt was made to determine the source and use of mucilage. Both of these points have been discussed in the literature and various views stated, some of which will be taken up a little later on in this paper. I was especially interested in trying to find whether or not it was used as food in this plant. Some of the drawings included are quite like those published by Pammel (13) and are used here only to help complete the set. Along with this study I attempted to find why the percentage of germination of the seeds was so low in nature, and with these studies made a few anatomical observations.

SEED PODS.

The seeds of *Gymocladus canadensis* Lam. are borne in a typical leguminosæ pod (Pl. XXIX, Fig. 1), which is from 3 to 7 inches long, to 2 inches wide and about 1 inch thick. The pods mature about the first of October and some may fall then, but the greater part of them hang on the trees well into spring, and it is not unusual to see some still hanging on the trees in midsummer.

The seeds, except in rare cases, remain in the pods until they fall and are broken up or decay, which may take two years.

The two valves of the pod are composed of a hard tissue which is made of cellulose and lignified cell walls. The very outer layer is cutinized and just under this there is a thin layer which gives a good test for tannin when treated with ferric chloride.

In the mature pods microchemical tests failed to show any reserve food in the form of starch, sugar, protein reserve cellulose, oil, inulin, or mucilage.

Within the two valves and surrounding the seeds is a loose cellular structure made of long cylindrical cells. (Pl. XXIX, Figs. 4, 5.) This mass of cells fills in the entire cavity around the seeds until they are nearly mature. Just before maturity these cells have a large amount of starch stored in them. (Pl. XXIX, Fig. 5.) About the time of maturity there is a general breaking down of this cellular structure into a brown sticky mass which remains around the seeds. After this disintegration the starch content of the cells disappears almost entirely (Pl. XXIX, Fig. 4), but for all I can tell from the available material there is no breaking down of the cell walls. Cellulose was the only membrane substance identified in the cell walls, except possibly pentosans, for which I got a slight test with orcin and hydrochloric acid. This substance absorbs water and swells some but not as much as mucilage does. It is sweet and with phenolhydrazine hydrochloric I was able to identify glucose and fuctose, and got two different tests for lactose.

The mucic acid test for galactose was negative. Tests for protein, reserve cellulose, oil, inulin and mucilage were all negative. Tannins were not found in this mass.

When the pods become moist they are often pushed open by this gummy mass, but if an excess of moisture is present the two valves will usually close tight. Fungi seem to thrive in this gummy mass, which may have some connection with germination of the seeds. I will take this up later.

Millspaugh reports this mass of cells, in the unripe fruit, and the green leaves as containing cytisine ($C_{24}H_{27}N_3O$) which is extracted with alcohol and used some in medicine. Millspaugh also reports the crushed green leaves as being an excellent fly poison when sprinkled with water and molasses. (See Millspaugh, l. c.)

Bretschneider (4) reports that the Chinese use this mass from the ripe pods as a soap, and he also lists it as one of the important articles of export from China. It does have a soapy consistency and lathers freely in water.

Insects are quite commonly found feeding upon this substance while in the pods.

STRUCTURE OF SEEDS.

There are from 1 to 7 seeds borne in each pod with an average of 4½ seeds in 200 pods examined. These seeds are attached to the pod with a funiculus which is about 9 mm. long and 1 mm. in diameter.

When dry the funiculus of mature seeds is very hard. In cross

section it has very thick-walled cells with a small cell cavity. (Pl. XXXII, Fig. 1.) The cell walls give a good test for cellulose but not test for lignin. After being soaked in water the outer layers of cells start swelling and at six hours they were rounded out as in Plate XXXII, Figure 2. Plate XXXII, Figure 5, shows the condition after 24 hours soaking. Here the cell walls have entirely broken down into a mucilaginous mass, leaving only the small masses of protoplasm intact. Some of the cells do not undergo this change, but only swell slightly and still retain their identity as cells. When these cells are converted into this mucilaginous substance there is a limit as to how far the reaction may go. Never have I found them going into a colloidal solution. After two days, when nearly all of the cells were in that state, the funiculus would still be tough enough to section readily. A good mucilage test was obtained in all stages of the swelling.

The only use that I could imagine for this phenomenon in the funiculus is that probably this mucilaginous layer could hold water in contact with the cutinized layer long enough for it to break through into the embryo. I do not have any experimental evidence to base this on.

Microchemical tests failed to show any form of stored food in the funiculus.

The seeds average about 16 mm. long, 14 mm. wide, and 11 mm. thick. (Pl. XXXIII, Fig. 1.) The average weight for 100 seeds was 2.3 grams. When mature, and fresh, they are of a glossy dark-brown color. Later, after they have laid on the ground for some time, they lose the glossy finish. This is due to a checking of the very outer layer of the epidermis. (Pl. XXX, Fig. 1, A-1.) I have never found the checks extending down through the entire epidermis. The seeds are so hard that the Chinese coolies strung them and used them for armor (5).

SEED COATS.

The hard seed coats are made up of two distinct layers. (Pl. XXX, Fig. 1.) The outer layer is the epidermis and is made up of several layers of very thick-walled, elongated cells, and averages about 0.2 mm. in thickness. (Pl. XXX, Fig. 2.) These cells were separated out by boiling in a concentrated solution of potassium hydroxide.

This outer layer forms a waterproof coat over the entire seed, there being a like layer laid down with the abscis layer, which cuts the seed off from the funiculus. This coat is so thoroughly water-

proofed that seeds showed no signs of swelling after being soaked in water for nine months. No weights were taken. Seed from the *Gleditsia triacanthos* L., which are waterproofed with a similar layer, only much thinner, showed no signs of swelling after being soaked for thirteen months.

The cells making up the epidermis give a good test for cellulose but not for lignin. In Plate XXX, Figure 1, the area A-1 gave a good test for chitin. This was checked up with insect wing and elm wood, but I still feel that there is possibly some other substance present which gave the reaction. This also dissolved out in boiling concentrated potassium hydroxide, but boiling saturated potassium hydroxide did not affect it. The area A-2 gives a strong test for cutin, and A-3 a much weaker test for cutin. Between the areas A-1 and A-2 (Pl. XXX, Fig. 1) there is a clear line which I was unable to identify. Various explanations of this line have been offered, but I did not try to check them up. Pammel (12) states that as far as he knows it is always connected with a reproductive body, and obviously has some function. I was unable to demonstrate any living protoplasm, or any form of storage substances, in fresh sections of the epidermis.

It is possible for seeds to be absolutely impervious to water and still carry on respiration, as O_2 can pass through a cutinized layer that is absolutely waterproof. A saturated solution of chromic acid and concentrated solutions of nitric, hydrochloric and sulphuric acids make their way through the seed coat after standing for some time. (See germination charts on pages 343 to 346.) The point of attack is usually at the point where the funiculus was attached. In most cases the seed coat was unaffected at any other point. Seeds that were in 20 per cent hydrochloric acid for two hours showed a general breaking down of the outer uncutinized area of the epidermis, but no damage was done to the cutinized part of the epidermis. Seeds soaked two hours in 50 per cent nitric acid were coated with a slimy mass formed by the breaking down of the uncutinized part of the epidermis. In the tables the seeds which germinated, after being treated with dilute acids, very likely had slight defects in the seed coats.

When this epidermal layer is broken the seed takes up water very rapidly and the epidermis cracks and peels off. (Pl. XXXIII, Fig. 2.)

The inner layer of the seed coat is made up of thick-walled cells (Pl. XXX, Fig. 1-B) which are very hard. There is no difference in

the structure or properties of these cells, except for the very outer layer where the cells are much different in shape (Pl. XXX, Fig. 1) but not in other general properties, and are apparently unaffected by the peeling off of the epidermis. These cells are connected to each other by relatively large pits in their walls, but the intercellular spaces are very small and few.

Microchemical tests for starch, reserve proteins, reserve or hemicellulose, oil, tannins, mucilage, and inulin were all negative. When boiled with Fehling's solution the seed coats gave a good reduction test for sugar. However, I cannot say that it was sugar, because it would not dissolve out in water, even after long soaking. It is evidently some carbohydrate which is easily hydrolized, or some other reducing substance, possibly an aldehyde, which is not soluble in water.

When the epidermis is cut through and the seeds then put into water for 48 hours there is a large amount of swelling in the seed coat. This swelling increases the thickness of the seed coat and also increases the circumference of the seed coat. The following weights and measurements are very close to an average:

Weight of seed dry	2.140 grams
Weight of seed soaked	7.400 grams
Water absorbed	5.292 grams

The seed coat had not yet broken as it did later.

Length of seed dry	18 mm.
Width of seed dry	16 mm.
Thickness of seed dry	11.4 mm.
Length of seed soaked	26.5 mm.
Width of seed soaked	22.7 mm.
Thickness of seed soaked	18 mm.
Thickness of seed coat dry	.6 mm.
Thickness of seed coat soaked	1.3 mm.

This last measurement was made on sections soaked on a slide, but the increase in thickness averages up pretty well with that which occurs in the whole seeds.

The walls of these cells are composed of cellulose and lignin. I was unable to detect any mucilage or other substances that would account for so large an amount of swelling. After swelling, the inner layer of the seed coat, which is the only part left, is very tough and pliable and will give to the pressure of the expanding cotyledons and mucilage within.

There is an exception to the above description of the inner layer

of the seed coat. At the point just under the abscis layer (Pl. XXIX, Fig. 3-H) the cells, when dry, appear just like all of the other cells of the inner layer (Pl. XXXII, Fig. 1), but react much differently. These cells give a test for cellulose but not for lignin. When put into water the walls of most of them start swelling, as in Plate XXXII, Figure 5, and continue swelling until they lose their identity as cell walls and become a mass of mucilage. This is a remarkable modification of the seed to aid in germination, as it is at this point that the growing radicle, which is the first part to emerge, must break through the seed coat. (Pl. XXIX, Fig. 3-H.) With these cells breaking down into a mucilaginous mass the tender radicle can much more easily force its way through. The swelling at this point is also much greater, the seed coat increasing from 7 mm. to 4 mm. in thickness. This mucilaginous substance, while being softer than that formed in the funiculus, will not go into a colloidal solution, but does give a good color test for mucilage.

MUCILAGE LAYER AROUND EMBRYO.

Just outside the seed coat is a hard, dry mass of substance (Pl. XXX, Fig. 1-C) which is white in color and composed of cells (Pl. XXXI, Fig. 1-A) and an amorphous mass which is made up of empty cell walls embedded in mucilage. Pammel (12) terms this endosperm. This layer is about 1 mm. thick and extends around the entire embryo, with a few exceptions. However, it is always much thicker on the sides than around the edges of the cotyledons. The cells, which remain intact, are covered with mucilage and form a loose cellular structure with large intercellular spaces. (Pl. XXXI, Fig. 1-B.) This structure or tissue is usually to the outer side next to the seed coat. This layer absorbs large amounts of water and is capable of swelling from 1 mm., the thickness when dry, up to 18-20 mm. in thickness when soaked over night. (Pl. XXXIII, Fig. 4.) The cellular structure usually remains intact and as the mucilage swells it is forced into the intercellular spaces and then on out of the cellular tissue. The cotyplasm of these cells, which are undoubtedly dead, gives only a slight test for proteins and in some cases it seemed to disintegrate and disappear. Very likely it is in a state of decomposition. The walls give a good test for cellulose but not for lignin.

The empty cell walls, which are in the amorphous mass, are composed of cellulose, and may or may not surround some protoplasm, usually not. (Pl. XXXI, Fig. 5.) Before maturity these cells and the cells remaining intact contained large amounts of starch which

disappeared during the ripening of the seeds. Whether this starch is converted into mucilage or not I cannot say, but it is quite evident that the mucilage is formed during the ripening of the seeds, and is not a disintegration product of the cell walls, taking place as the cells absorb water, because there is no reduction in the thickness of the cell wall. (Pl. XXXI, Figs. 1-B and 4-B.) The mucilage found here will lose its identity and pass into a colloidal solution. I say colloidal because it will not pass through a celloidon dializing tube and it does not exert any osmotic pressure. The swelling of this mucilage, together with the swelling of the embryo, is often enough to break the seed coat even when the radicle is not trying to break through. (Pl. XXXIII, Fig. 3-B.)

This mucilage also absorbs water and conducts it to the entire outer surfaces of the cotyledons and in this way hastens germination. Also, the amount of water held by this mucilage is enough to tide the germinating seedlings over a temporary dry spell. While it is helping in germination in this way, it is hindering it in another because of its excellent qualities as a culture medium for injurious organisms. During germination this mucilage contains considerable sugar, which is undoubtedly drawn from the cotyledons. From this we might conclude that the substance previously noted in the seed coat during germination and giving the reducing test for sugar, might be sugar diffusing out from the cotyledons or a hydrolisis product of the seed coats being conducted inward. However, as before stated, the seedlings grew just as well in the laboratory with this mucilage removed as they did with it present. This was not checked up with field experiments. This was noted in the laboratory; when the food was all removed from the cotyledons there were no reducing substances left in the mucilage.

Experiment in which the seed coats were removed with the mucilage layer left intact, showed that there was no loss in dry weight, from the mucilage, during germination and development of the seedlings as long as they would grow on the food stored in the seeds. In these experiments the mucilage layer was removed and weighed dry. They were then replaced and the seeds put to germinate on cotton, in bottles. When growth had ceased the plants were carefully removed and all traces of mucilage washed back into the bottles. The bottles, with the mucilage and cotton, were dried and weighed but in no case was there any loss in weight. Furthermore, seeds germinated and grew just as well and long without this mucilage layer as they did with it present.

Upon being hydrolized with 4 per cent sulphuric acid and then

neutralized with barium carbonate, there was a good test for glucose, fructose and possibly mannose. When soaked over night in 10 per cent lead acetate the mucilage absorbed some water but would not swell any more when put into pure water. With alcohol it reacts slightly different from the mucilage found in the bark of *Ulmus fluva* in that it does not absorb water from 60 per cent alcohol and is precipitated by 70 per cent alcohol. The mucilage from *Ulmus fluva* absorbs water from 60 per cent alcohol and is not affected by 70 per cent alcohol.

In the work done by others there are many theories advanced as to the origin and function of mucilage. Edna L. Smith quotes Czapek's[1] summary on the origin and function of mucilage in plants:

1. Secondary thickenings in the cell walls. It may exude from the cell upon the epidermis or into the intercellular spaces.
2. Specialized layer between cell wall and cytoplasm.
3. Arises in the protoplast.

Czapek lists the protoplast slime as water holders, and that found within the tubers of orchids and the endosperm of legumes as reserve carbohydrates. He considers that slime membranes are never used as stored food, but rather as serving to (*a*) imbibe and hold water during germination and to protect against excessive transpiration, (*b*) anchor seeds to substratum, (*c*) facilitate gliding of the seeds, and (*d*) protect water plants from their natural enemies. In summarizing her work, Miss Smith concludes that the mucilage in the floral organs of *Aspasia* sp. and *Oncidium stipitata* must originate in the living protoplasm.

Gregory (8) lists the sources of mucilage as (*a*) the absorption of water by cellulose walls, as in the epidermis of flax, and (*b*) secretion by special glands. She attributes two uses to this mucilage, (*a*) against excessive water and drouth in the lower members of the *Hepaticæ,* and (*b*) protection of the embryo within the open archegonia of many of the *Musci,* from the water. No doubt she referred to the mechanical effects of the water currents. Gregory also lists gums as decomposition products of the cell walls, and in *Ricciæ,* she states that the origin of the mucilage is unknown.

Pfeffer (9) reports the gelatinous layer of *Nostoc* as being able to absorb water in the ratio of 200-1. He also states that mucilage may be converted into cellulose by the proper treatment with acid, but he does not explain the reverse reaction.

Sachs (10) gives the cellulose walls as the source of mucilage and

1. Die Chemie und Biologie der Planzensekrete, Leipsig, 1903 and 1921.

states that the walls or parts of walls which turn into mucilage are more refractive than the walls which remain intact. In the endosperm of *Ceratonia siliqua* the outer layers of the cell walls become mucilage and the inner layers remain as a highly refractive system. In the linseed and quince seed the inner thickened mass of the epidermis changes to mucilage and absorbs enough water to burst the cuticle. Sachs offers no explanation of the chemical changes which take place in the conversion of cellulose to mucilage.

In my work I found conclusive evidence that in the funiculus and in the layer of cells in the seed coat just below the funiculus, the mucilage is formed by the absorption of water by the cell walls. That is, these walls when dry gave a reaction for cellulose and then when wet behaved like mucilage. This change may have taken place at some previous time, providing that there is some substance left that will give the cellulose reaction. With the material available it was impossible to determine the source of the mucilage around the embryo, but there was proof that it did not develop from the remaining cell walls by the absorption of water. It probably originated about the time of maturity and it may be, as Pammel (12) suggested, formed by the absorption of water by secondary thickenings in the walls. I feel sure that the large starch content that was present just before maturity played an important part in the formation of this mucilage.

EMBRYO.

The cotyledons of the embryo are very hard and swell considerably when soaked in water. The outer epidermis is covered with a very thin film of cutin. (Pl. XXXIV, Fig. 3-F.) The inner epidermis (Pl. XXXIV, Fig. 3-D) is not cutinized, and the two cotyledons are grown together for the greater part of the way at the very thick outer walls of the epidermal cells. (Pl. XXXIV, Fig. 3-E.) A general drawing (Pl. XXIX, Fig. 3) shows the relative size, shape and position of the different parts of the embryo.

The cotyledons contain starch, and oil as reserve food, and I got a faint test for inulin with the α naphthol-hydrochloric acid test, but was unable to detect any inulin crystals. There was no mucilage, reserve cellulose or tannin found.

GERMINATION OF SEEDS.

During the germination the cotyledons remain in the ground and do not separate at all. There is enough food stored within them to support the seedling until it gets its second pair of leaves. The first

leaves developed on the seedlings are once pinnate instead of twice pinnate as they are in mature trees.

Plate XXXV, Figures 1, 2 and 4, are drawings of bleached, embryonic leaves taken from the buds of mature trees. Figures 1 and 2 show the differentiation of the cells to form the water tubes of the leaf. Figure 1 shows water tubes developing out in the tissue of the leaf without any water tubes connecting back to the main vein already developed. Figure 2 shows the cell elongated to form water tubes but with the thickening of the walls beginning at the point farthest from the main vein. This is like the development of the veins making up the circulatory systems of animals. In young seedlings that have developed very rapidly this method of vascular development is very pronounced, much more so than in the mature trees.

Plate XXXIV, Figure 4, shows the development of the vascular bundles following closely behind the growing tip of the leaves. Epidermal cells were differentiating to form stomata at a point midway between A and B. The leaves on the mature trees are covered with hairs (Pl. XXXIV, Fig. 4-C), but in the young seedling very few are found. (Pl. XXXV, Fig. 1.) The bleached, mature leaf, looking up from the bottom, shows epidermis, veins and spongy parenchyna with relatively small intercellular spaces, much smaller than those in the palisade prenchyma. (Pl. XXXVI, Fig. 2-E.) In cross sections (Pl. XXXVI, Figs. 1, 2) the structure is quite like other leaves. The edges of the leaves have a much heavier coat of cutin than is found on the general surface or at the midrib. (Pl. XXXVI, Figs. 1-A and E, and Figs. 2-A and E.) The edges of the leaves are also strengthened with a group of thick-walled cells. (Pl. XXXVI, Fig. 1-F.) The epidermal cells at this point are also much heavier. At the midrib and the larger lateral veins there is a large amount of fibrous tissue developed for strength. (Pl. XXXVI, Fig. 2-I.)

GERMINATION OF SEEDS IN NATURE.

As best I can learn, germination of these seeds is rather uncommon in nature. On our home place there were two large and one small *Gymocladus canadensis* Lam. trees which bore many seeds each year, but during the ten years 1900-1910 only two young trees appeared, and they grew up from the exposed roots of one of the larger trees. In the winters of 1910 and 1911 the timber was burned over, killing many acres of the trees. Now the *Gymocladus canadensis* Lam. is the predominating tree, the seeds having been well

scattered by the neighborhood children who used them in their sling shots.

There is another similar case a few miles northwest of Lawrence where a fence row is well seeded with *Gymnocladus canadensis* Lam. The farmer owning the land said that they appeared soon after he started burning the fence row to kill the chinch bugs. The fence separated a cultivated field and wooded pasture along a creek. The *Gymnocladus canadensis* Lam. which furnished the seeds stood about 50 feet from the fence, but there were no young trees except along the fence row where the burning was done.

Another still more striking example showed up on the campus in the spring of 1920. While burning the grass on the campus, in early spring, the fire burned around five *Gymnocladus canadensis* Lam. trees along the north edge of Marvin's grove. When the weather got warm hundreds of seedlings sprang up under these five trees while under the others there would be from none, the usual condition, up to three which were found only under one tree, where the grass was not burned.

Attempts to germinate the seeds without some form of special treatment, which will be taken up later, were useless except in the case of some seeds that had defective seed coats. (Pl. XXIX, Fig. 2.) In 10,000 seeds examined 30 were found with such coats. Some of these would have a break in the cutinized epidermis only, while in others the break would extend down into or even through the woody tissue which makes up the greater part of the seed coat. The five shown in Plate XXIX, Figure 2, were all taken from one pod. Usually there was but one in a pod. I could not find any cause for these seeds having defective coats. There were no marks on the pod nor any other signs of insects having used the pods as a host plant. it seems to be just a case of the tissue not developing. It was not possible to get enough of these seeds to make any extensive study upon them. With these defective seeds I got 100 per cent germination in the only test I ran, using 20 seeds. (See table 36.) Pammel and King (13) reported 80 per cent germination on seeds buried 3½ feet under ground for 6½ years and about the same percentage of germination with seeds 7 months old. They do not mention any special treatment before planting.

Sargent (11) reports that *Gymnocladus canadensis* Lam. may be propagated from seeds which will germinate in the second or third year. My results with seeds known to be two years old did not

show a satisfactory per cent of germination. He gave the best method for propagation to be from root cuttings.

The treatment to aid and hasten germination falls under two general methods, physical and chemical, but the thing accomplished is the same in both cases, namely, to break through the impermeable epidermis of the seed coat and allow water to enter.

The first treatment undertaken was chemical and consisted in soaking the seeds in different concentrations of nitric, sulphuric, and hydrochloric acid. With this I got varied results. The tables show that the year-old seeds were much more resistant to the action of acids than the two-year-old seeds. Where the higher concentration of acids was used for a longer period the germination was low on account of the acid getting through to the embryo within and killing it. Where there was some germination with the weaker concentrations of acids, it is evident that the seed coats were not so well developed, or else there was some small defect in the waterproof epidermis. These experiments, tables 30-49, were carried on indoors where conditions were very unfavorable for plant growth, and a seed was considered as germinated when the radicle had developed enough to break through the seed coat. In this work the seeds were planted in sterilized petri dishes.

The physical treatment of the seeds consisted in destroying a part of the epidermis by either filing a hole in it or burning part of it away. The filing was a simple process requiring little time or care in handling. The burning was accomplished by putting the seeds on a coarse heavy screen and passing through the flame until the wires were red hot and then dropping them into water. Considerable overheating was required to kill the embryo, so that this method was very successful and much quicker than filing. Disinfectants were used with these seeds to kill the spores which were always present in the gummy mass adhering to the seeds. In the acid-treated seeds the disinfectants were not considered necessary. Where disinfectants were used on seeds either filed or burned there was a higher percentage of germination than with seeds with similar treatment but not disinfected. Additional tests were run on these seeds by soaking some of them 48 hours in water before planting. This gave a higher percentage of germination than when the seeds were not soaked. With this type of treatment the results were about the same with seeds filed or burned, and the best results were obtained by those that were soaked for 48 hours and then disinfected before planting.

SPRING, 1921.

OUTDOOR TESTS—SECOND-YEAR SEEDS.

(1) Burned, soaked 48 hours in water, treated 15 minutes with 1-1000 $HgCl_2$, and washed thoroughly in sterilized water.
Planted April 30.....May 20.... 46 germinated.

(2) Burned, soaked 48 hours in water, treated 15 minutes with 20 per cent formaldehyde and washed in sterilized water.
Planted April 30..... May 20.... 39 germinated.

(3) Burned, soaked 48 hours in sterilized water.
Planted April 30..... May 20.... 37 germinated.

(4) Filed, soaked 48 hours in water, treated 15 minutes with 1-1000 $HgCl_2$ solution and washed with sterilized water.
Planted April 30..... May 20.... 32 germinated.

(5) Filed, soaked 48 hours in water, treated 15 minutes with 20 per cent formaldehyde and washed with sterilized water.
Planted April 30..... May 20.... 40 germinated.

(6) Filed and soaked 48 hours in sterilized water.
Planted April 30..... May 20.... 19 germinated.

(7) Treated with 50 per cent HCl for 30 minutes and washed in sterilized water.
Planted April 30..... May 20.... 17 germinated.
May 31.... 47 germinated.

(8) Treated with 50 per cent HCl for 30 minutes and soaked 48 hours in sterilized water.
Planted April 30..... May 20.... 13 germinated.

(9) Burned, treated with 1-1000 $HgCl_2$, and washed in sterilized water.
Planted April 30..... May 20.... 23 germinated.
May 30.... 26 germinated.

(10) Burned, treated 15 minutes with 20 per cent formaldehyde and washed.
Planted April 30..... May 20.... 26 germinated.
May 30.... 35 germinated.

(11) Burned.
Planted April 30..... May 20.... 2 germinated.
May 30.... 21 germinated.

(12) Filed, treated 15 minutes with 1-1000 $HgCl_2$ and washed in sterilized water.
Planted April 30..... May 20.... 17 germinated.
May 30.... 21 germinated.

(13) Filed, treated 15 minutes with 20 per cent formaldehyde and washed in sterilized water.
Planted April 30..... May 20.... 23 germinated.
May 30.... 30 germinated.

(14) Filed.
Planted April 30..... May 20.... 12 germinated.
May 30.... 13 germinated.

OUTDOOR TESTS—FIRST-YEAR SEEDS.

(15) Burned, soaked 48 hours in water, treated 15 minutes with 1-1000 $HgCl_2$ solution and washed thoroughly in sterilized water.
Planted May 1..... May 2.... 46 germinated.

(16) Burned, soaked 48 hours in water and treated 15 minutes with 20 per cent formaldehyde solution.
Planted May 1..... May 2.... 43 germinated.

(17) Burned, soaked 48 hours in sterilized water.
Planted May 1..... May 21.... 33 germinated.

(18) Filed, soaked 48 hours in water, treated 15 minutes with 1-1000 $HgCl_2$ solution and washed with sterilized water.
Planted May 1..... May 21.... 47 germinated.

(19) Filed, soaked 48 hours in water, treated 15 minutes with 20 per cent formalin and washed in sterilized water.
Planted May 1..... May 21.... 38 germinated.

(20) Filed, soaked 48 hours in sterilized water.
Planted May 1..... May 21.... 21 germinated.

(21) Treated with 50 per cent HCl for thirty minutes and washed in sterilized water.
Planted May 1..... May 21.... None germinated.

(22) Treated with 50 per cent HCl for thirty minutes, and soaked 48 hours in sterilized water.
Planted May 1..... May 21.... 3 germinated.

(23) Burned, treated 15 minutes with 1-1000 $KgCl_2$ solution and washed in sterilized water.
Planted May 1..... May 21.... 23 germinated.
May 31.... 40 germinated.

(24) Burned, treated 15 minutes with 20 per cent formaldehyde solution and washed in sterilized water.
Planted May 1..... May 21.... 25 germinated.
May 31.... 27 germinated.

(25) Burned and washed in sterilized water.
Planted May 1..... May 21.... 3 germinated.
May 31.... 12 germinated.

(26) Filed, treated 15 minutes with 1-1000 $HgCl_2$ and washed in sterilized water.
Planted May 1..... May 21.... 24 germinated.
May 31.... 29 germinated.

(27) Filed, treated 15 minutes with 20 per cent formaldehyde solution and washed in sterilized water.
Planted May 1..... May 21.... 11 germinated.
May 31.... 14 germinated.

(28) Filed.
Planted May 1..... May 21.... 8 germinated.
May 31.... 21 germinated.

SEEDS DUG UP FROM UNDERGROUND.

(29) The exact age was unknown, but some of them must have been several years old. Burned and soaked 48 hours and planted in sterile bottles. 200 seeds planted.
Planted March 20..... April 1.... 168 germinated.

INDOOR TESTS—FIRST-YEAR SEEDS.

(Fifty seeds used in each test.)

(30)	Treated with 50% HCl for one hour	20 germinated.
	Treated with 50% HCl for two hours	47 germinated.
	Treated with 50% HCl for three hours	43 germinated.
(31)	Treated with 75% HCl for 10 minutes	45 germinated.
	Treated with 75% HCl for 30 minutes	46 germinated.
	Treated with 75% HCl for 1 hour	28 germinated.
	Treated with 75% HCl for 2 hours	20 germinated.
(32)	Treated with 100% HCl for 5 minutes	3 germinated.
	Treated with 100% HCl for 20 minutes	30 germinated.
	Treated with 100% HCl for 45 minutes	1 germinated.
(33)	Treated with 50% HNO_3 for 1 hour	0 germinated.
	Treated with 50% HNO_3 for 2 hours	0 germinated.
	Treated with 50% HNO_3 for 3 hours	0 germinated.
(34)	Treated with 75% HNO_3 for 10 minutes	0 germinated.
	Treated with 75% HNO_3 for 30 minutes	1 germinated.
	Treated with 75% HNO_3 for 1 hour	15 germinated.
	Treated with 75% HNO_3 for 2 hours	0 germinated.
(35)	Treated with 100% HNO_3 for 5 minutes	1 germinated.
	Treated with 100% HNO_3 for 20 minutes	11 germinated.
	Treated with 100% HNO_3 for 45 minutes	0 germinated.
(36)	Seed coats filed	48 germinated.
	Seed coats burned	49 germinated.
	Seeds with holes in coats (only 20 available) (Pl. XXIX, Fig. 2.)	20 germinated.
(37)	Treated with 50% H_2SO_4 for 1 hour	1 germinated.
	Treated with 50% H_2SO_4 for 2 hours	0 germinated.
	Treated with 50% H_2SO_4 for 3 hours	5 germinated.
(38)	Treated with 75% H_2SO_4 for 10 minutes	0 germinated.
	Treated with 75% H_2SO_4 for 30 minutes	5 germinated.
	Treated with 75% H_2SO_4 for 1 hour	10 germinated.
	Treated with 75% H_2SO_4 for 2 hours	11 germinated.
(39)	Treated with 100% H_2SO_4 for 5 minutes	3 germinated.
	Treated with 100% H_2SO_4 for 20 minutes	25 germinated.
	Treated with 100% H_2SO_4 for 45 minutes	17 germinated.
(40)	Treated with 50% HCl for 1 hour	28 germinated.
	Treated with 50% HCl for 2 hours	45 germinated.
	Treated with 50% HCl for 3 hours	17 germinated.

INDOOR TESTS—SECOND-YEAR SEEDS.

(41)	Treated with 75% HCl for 10 minutes	36 germinated.
	Treated with 75% HCl for 30 minutes	38 germinated.
	Treated with 75% HCl for 1 hour	12 germinated.
	Treated with 75% HCl for 2 hours	0 germinated.
(42)	Treated with 100% HCl for 5 minutes	20 germinated.
	Treated with 100% HCl for 20 minutes	6 germinated.
	Treated with 100% HCl for 45 minutes	0 germinated.
(43)	Treated with 50% HNO_3 for 1 hour	3 germinated.
	Treated with 50% HNO_3 for 2 hours	5 germinated.
	Treated with 50% HNO_3 for 3 hours	9 germinated.

(44)	Treated with 75% HNO_3 for 10 minutes	1 germinated.
	Treated with 75% HNO_3 for 30 minutes	2 germinated.
	Treated with 75% HNO_3 for 1 hour	12 germinated.
	Treated with 75% HNO_3 for 2 hours	0 germinated.
(45)	Treated with 100% HNO_3 for 5 minutes	5 germinated.
	Treated with 100% HNO_3 for 20 minutes	9 germinated.
	Treated with 100% HNO_3 for 45 minutes	1 germinated.
(46)	Seed coats filed	47 germinated.
	Seed coats burned	41 germinated.
(47)	Treated with 50% H_2SO_4 for 1 hour	12 germinated.
	Treated with 50% H_2SO_4 for 2 hours	7 germinated.
	Treated with 50% H_2SO_4 for 3 hours	9 germinated.
(48)	Treated with 75% H_2SO_4 for 10 minutes	8 germinated.
	Treated with 75% H_2SO_4 for 30 minutes	27 germinated.
	Treated with 75% H_2SO_4 for 1 hour	21 germinated.
	Treated with 75% H_2SO_4 for 2 hours	17 germinated.
(49)	Treated with 100% H_2SO_4 for 5 minutes	0 germinated.
	Treated with 100% H_2SO_4 for 20 minutes	12 germinated.
	Treated with 100% H_2SO_4 for 45 minutes	11 germinated.

SUMMARY.

1. The mucilage in the funiculus and in the region just below the funiculus is formed by the absorption of water by the cell wall.

2. The mucilaginous mass around the seeds, and around the embryo within the seed, appears to be formed at some time attending the maturity of the seeds and follows closely the disappearance of the starch from these cells. These cells also retain their cell walls.

3. The mucilage formed may go into a colloidal solution as in the seed pods, surrounding the seeds, or in the region of the seed coat just beneath the funiculus, or in the mass surrounding the embryo. Also it may absorb a limited amount of water as in the funiculus.

4. The mucilage is not used as food.

5. Water tube may form in the developing leaves without being directly connected back to the veins by differentiated cells.

REAGENTS AND METHODS.

1. CELLULOSE. *Iodine.*[2] Place section in a drop of I_2KI solution. (Iodine 3 grams, and potassium iodide 1.5 grams in 100 cc. of distilled water.) Cellulose membranes swell and turn blue.

Chloride of zinc.[1] Mount sections in a drop of solution containing 14 grams of zinc chloride, 5 grams of potassium iodide and 0.89 gram of iodine in 20 cc. of water. This turns cellulose walls violet.

2. HEMICELLULOSE.[2] Mount sections on a slide in 3 per cent H_2SO_4 and heat on a water bath. From 10 minutes to 2 hours is required to give complete hydrolysis. Note thickness of membrane before and after heating.

3. CUTIN. *Sudan III.* Put sections, over night, in a solution containing 0.01 gram Sudan III, 5 grams of 95 per cent alcohol, and 5 cc. of glycerine. Cutinized and suberized walls are stained red by this solution.

4. CHITOSANS.[2] Put sections into boiling saturated solution of potassium hydroxide. Cover with a watch glass and boil for 30 minutes. Cool, neutralize with hydrochloric acid, wash in water and mount in I_2KI. Chitosans give a violet color.

5. GALACTANS. (See galactose.)

6. GLUCOSIDES.[1] *General method.* If there is no reduction of Fehling's solution, put the sections into fresh Fehling's solution and let stand over night in a 37-50° oven. If glucosides were present, there should be a reduction of Fehling's solution due to the hydrolysis of the glucosides.

7. INULIN. Put sections in 70 per cent alcohol for 2 to 4 days and sphaero crystals of inulin will separate out. To identify these, locate the crystals under the microscope and add a drop of 15 per cent α naphthol in alcohol, and then a drop of concentrated sulphuric acid. Inulin crystals become violet and dissolve, giving a violet color. This must be watched closely as sugars in solution will give the same color.

8. LIGNIN.[2] Mount section in a solution of phloroglucin (phloroglucin 0.1 gram in 10 cc. of alcohol) and let stand until the solution is partly evaporated and then add a drop of concentrated hydrochloric acid. Lignin gives a violet color.

9. MANNANS.[2] Put the section in a watch glass containing 2 per cent hydrochloric acid and heat gently, on water bath, for 30 minutes. Neutralize with 2 per cent ammonium hydroxide and test for mannose. (See sugars.)

10. OILS.[1] Put sections in Sudan III. (See cutin.) Oil appears as droplets and are stained red. Lipoids give the same reaction but they are insoluble in acetone.

11. PENTOSANS.[2] Treat the same as for lignin. Pentosans give a cherry-red color.

12. PROTEINS.[2] I_2KI.[2] A solution containing 1 gram of iodine, 3 grams potassium iodide, in 100 cc. of water, give a yellow precipitate with protein.

Millon's reagent.[1] Dissolve 20 grams of mercury in 20 grams of nitric acid and add 40 grams of distilled water. Proteids are colored brick red with this reagent.

Xanthoproteic.[2] Mount section in a drop of concentrated nitric acid. Proteins turn yellow. Add a drop of ammonium hydroxide and they then turn to a deep orange color.

13. STARCH.[2] Starch turns blue when treated with a I_2KI solution containing 0.3 gram of iodine and 1.5 grams of potassium iodine in 100 cc. of water.

14. SUGARS. Phenylhydrazine hydrochloride. (*a*) Phenylhydrazine hydrochloride 1 gram dissolved in 9 grams of glycerine. (*b*) Sodium acetate 1 gram dissolved in 9 grams of glycerine.

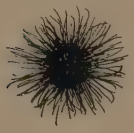
Keep both in brown bottles. Mix a drop of each on a slide and mount section in it.

(1) Fructose osazone crystals appear after 7 to 13 hours at 20° C. (See accompanying drawing.)

(2) Glucose osazone crystals appear after standing 2 days at 20° C. (See accompanying drawing.)

(3) Sucrose osazone crystals, like 1 and 2, appear after standing 2 days at 20° C. and then heating for 30 to 40 minutes.

(4) Mannose gives hydrozone crystals in a few minutes. (See accompanying drawing.)



Fructose osazone crystals.

Glucose osazone crystals.

Hydrozone crystals.

(5) Galactose.[2] Heat sections in evaporating dish containing 15 cc. concentrated nitric acid until it is evaporated to about 3 cc. Add 15 cc. of water, set away for 24 hours and mucic acid crystals will appear.

15. TANNINS.[1] Tannins give a blue color when treated with an aqueous solution of ferric chloride.

BIBLIOGRAPHY.

1. STEVENS, W. C. Plant Anatomy. Third edition.
2. ECKERSON, S. H. Laboratory Outline for Plant Microchemistry.
3. MILLSPAUGH. American Medicinal Plants. Homeopathic Remedies, i. 53, t. 53.
4. BRETSCHNEIDER. Notes on Some Botanical Questions Connected with the Export Trade of China. 14 Hanbury Science Papers.
5. NICHOLSON, GEORGE. The Soap Tree of China. Garden and Forest, Vol. II, page 139.
6. LIVINGSTON, B. E. Palladin's Physiology of Plants.
7. SMITH, EDNA L. The Histology of Certain Orchids with Reference to Mucilage Secretion and Crystal Formation. Bulletin of Torrey Botanical Club. Vol. 50, No. 1, Jan., 1923.
8. GREGORY, EMILY L. Plant Anatomy.
9. PFEFFER, W. Physiology of Plants.
10. SACHS, JULIUS. Botany.
11. SARGENT, CHARLES S. Silva of North America. Vol. III, page 69.
12. PAMMEL, L. H. Anatomical Characters of Seeds of Leguminosæ.
13. PAMMEL, L. H. AND KING, C. M. Germination of Some Trees and Shrubs and Their Juvenile Forms. Pro. Iowa Acad. Sci. 25; 292-340, 1920.

PLATE XXIX.

1. Mature seed pod. $\times$ ¾.

2. Seeds with defective seed coats. $\times$ ¾.

3. Cross section through entire seed. $\times$ 4½. A, Radicle. B, Hypocotyl. C, Epidermal coat. D, Woody layer of seed coat. E, Mucilage layer. F. Cotyledons. G, Veins running from cotyledons to growing points.

4. Cells taken from the gummy mass around the seeds in a mature pod. $\times$ 1000. A, Starch.

5. Cells taken from around the seed before the cells start disintegrating. $\times$ 1000. A, Starch.

PLATE XXIX.

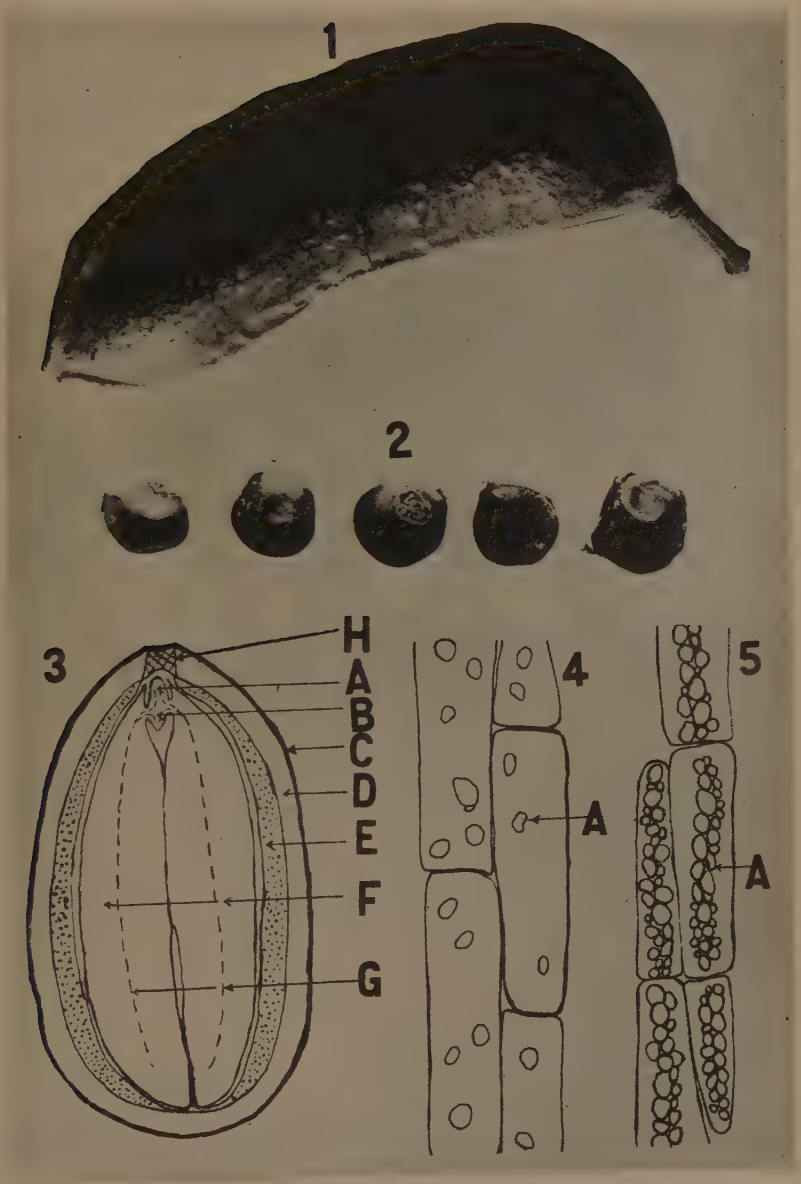


PLATE XXX.

1. Section of a soaked seed coat. × 110. A, Multiple epidermis: (1) area which gives a test for chitin; (2) Area which gives a strong test for cutin; (3) Area which gives a slight test for cutin. B, Woody cells which make up the body of the seed coat. C. Mucilaginous layer.
2. Cells taken from 1-A, 1. × 1000.
3. Cells taken from 1-B, dry. × 520.
4. Cells taken from 1-B, soaked. × 520.

PLATE XXX.

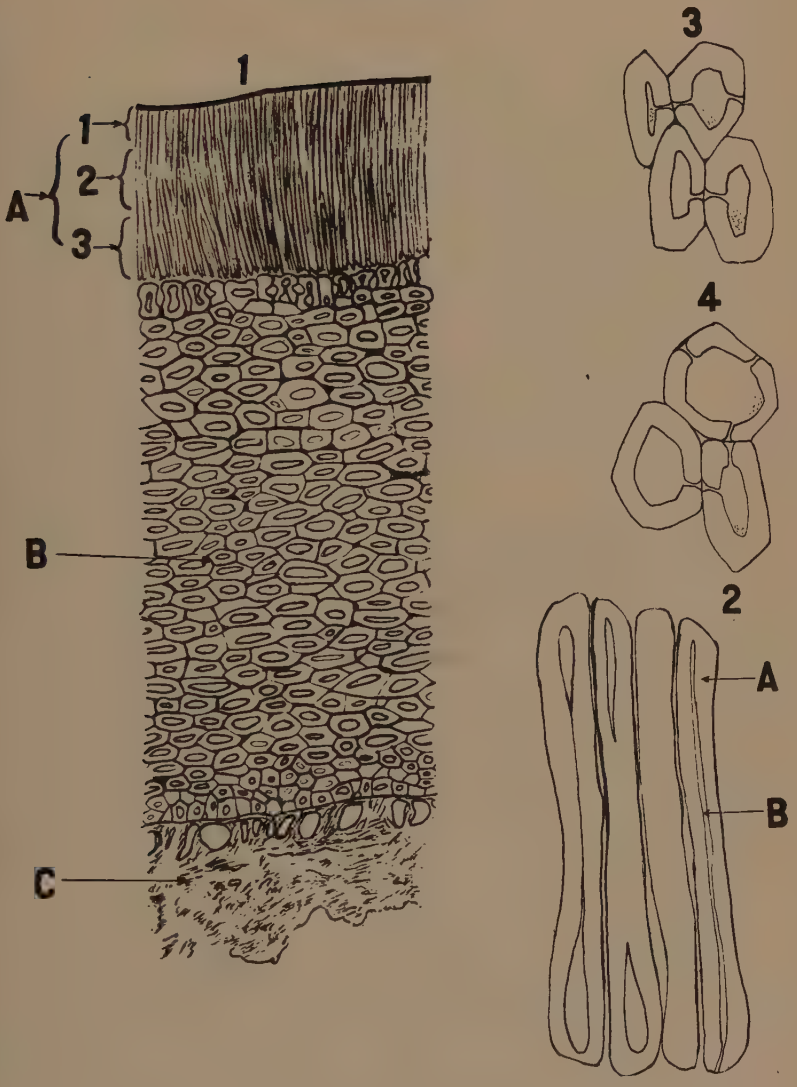


PLATE XXXI.

1. Cells from mucilage layer around the embryo. × 520. A, Cellulose wall. B, Intercellular space. C, Mucilage around cell. D, Space between cell wall and protoplasm.

2. Same section as 1, after soaking 6 hours. × 520.

3. Isolated cells, dry. × 1000. (Indexed as above.)

4. Same cells as in 3, after soaking 24 hours. × 1000. (Indexed as above.)

5. Section from amorphous mass of mucilage. × 520. A, Cellulose cell walls. B, Protoplasm with surrounding membrane.

PLATE XXXI.

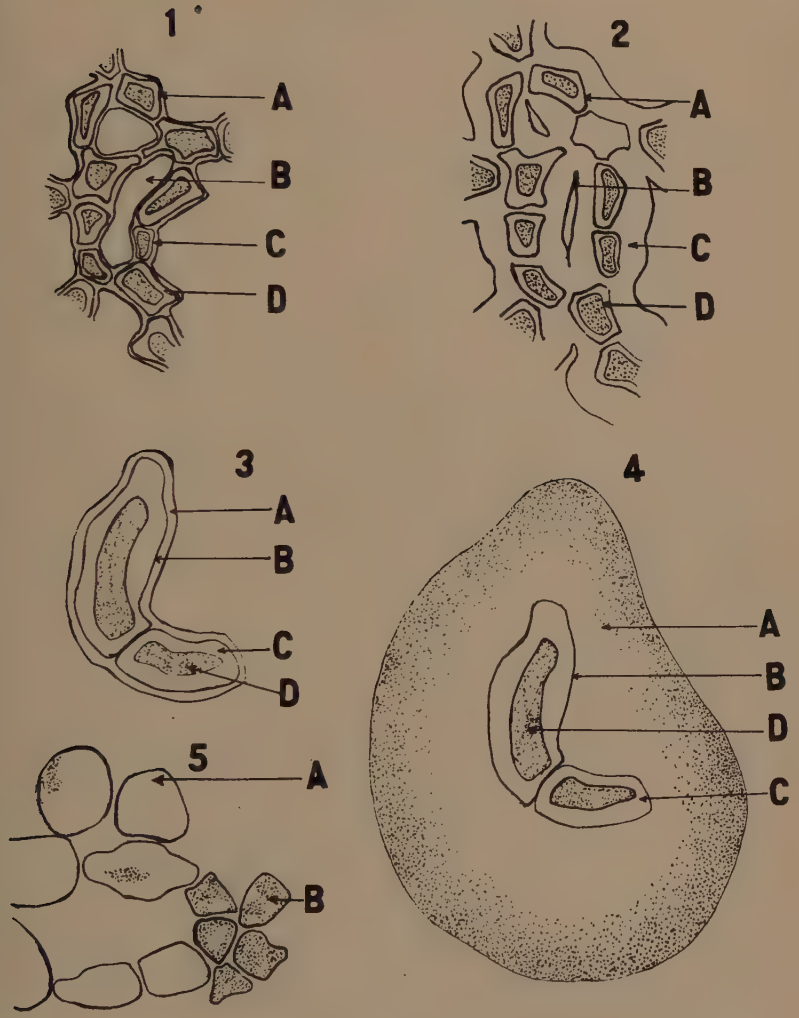


PLATE XXXII.

1. Cells from the funiculus. ×460. A, Cell wall. B, Protoplasm.
2. Same as 1, after 6 hours soaking. ×460. (Indexed as above.)
5. Same as 1, after 24 hours soaking. ×460.
4. Cells from beneath the funiculus. ×460.
3. Same as 4, after 24 hours soaking. ×460.

PLATE XXXII.

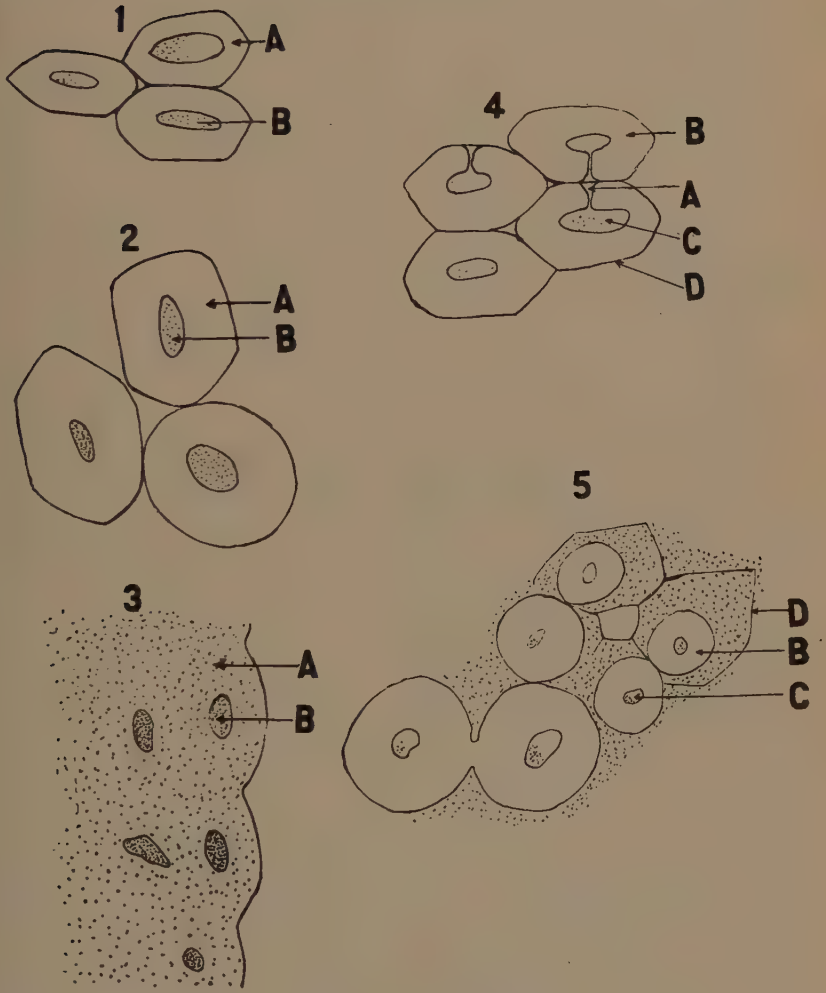


PLATE XXXIII.

1. Dry seed. × ¾.
2. Seed soaked 24 hours. × ¾. A, Epidermal layer peeling off.
3. Seed soaked 48 hours. × ¾. B, Mucilage mass.
4. Mucilage mass. × ¾. C, Soaked 24 hours. D, Cellular part. E, Mucilage mass dry.
5. First stage in germination. × ¾.
6. Second stage in germination. × ¾.

PLATE XXXIII.

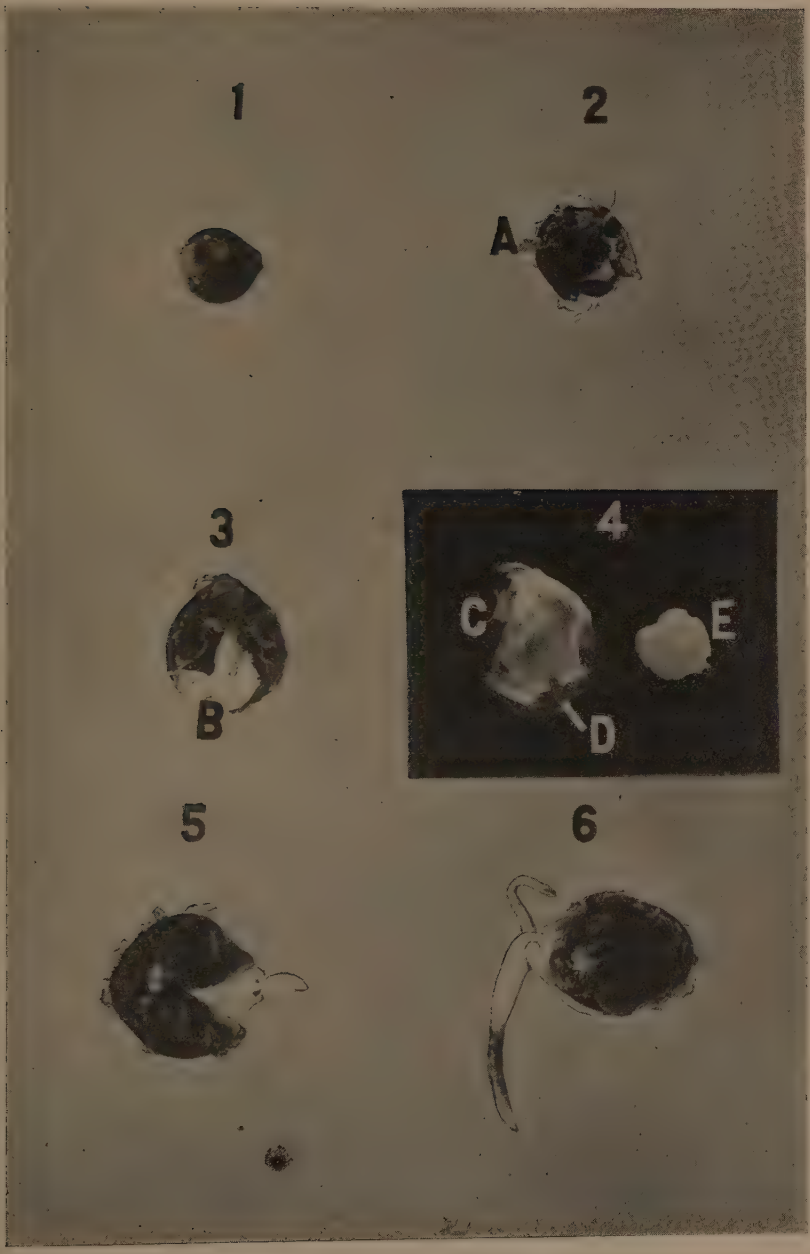


PLATE XXXIV.

1. Section of young leaflet from mature tree. ×280. A, Undifferentiated cells which later become water tubes.

2. Section of same leaflet. ×280. A, Cells which are differentiated but have not laid down the thickenings in the walls.

3. Section through cotyledon. A, Outer epidermis. F, A very thin cutinized layer. B, Cells. C, Intercellular space. D, Inner epidermis. E, Heavy wall which is not cutinized.

4. Section of a bleached leaflet tip. ×400. A, Epidermis. B, Water tubes. C, Hairs.

PLATE XXXIV.

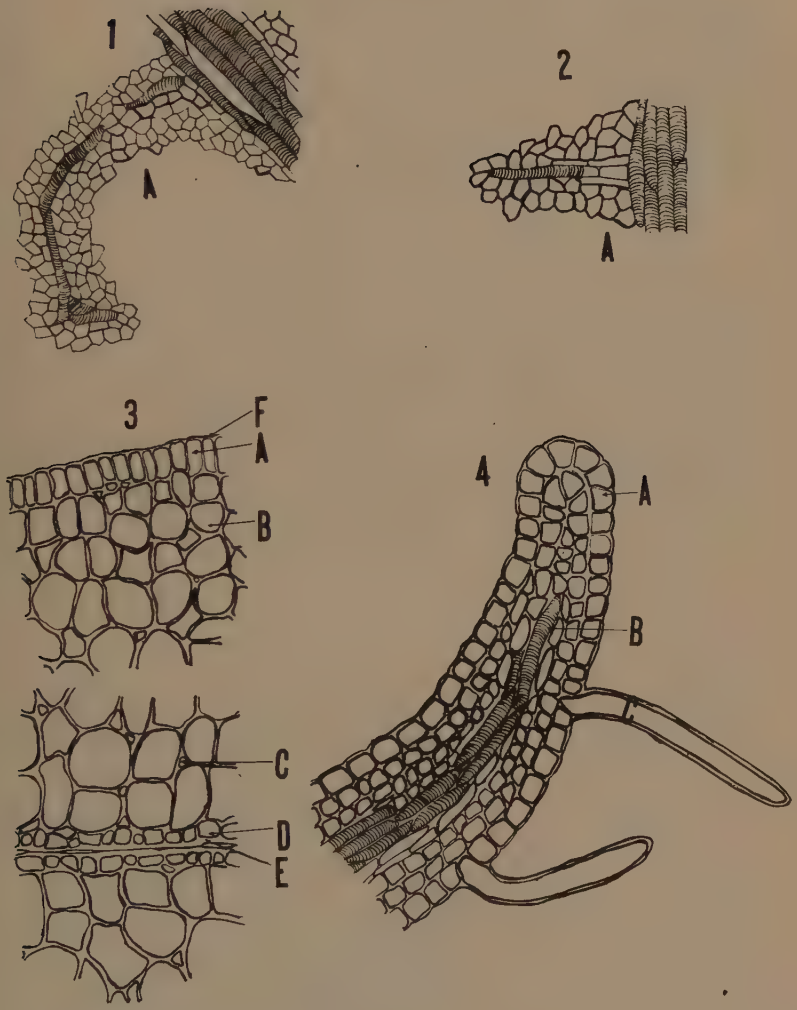


PLATE XXXV.

1. View of under side of bleached leaf. × 220. A, Hair. B, Vein. C, Spongy parenchyona. D, Stoma and ground cells. E, Cell wall of epidermis indicated with double line to differentiate between spongy parenchyma cell walls.

2. View of same leaf from above. × 220. B, Vein. C, Cell wall of epidermis. D, Palisade cells. E, Intercellular space between palisade cells.

3. Strip of lower epidermis with stomata. × 220.

4. Strip of upper epidermis. × 220.

(Nos. 3 and 4 were taken from old leaves.)

PLATE XXXV.

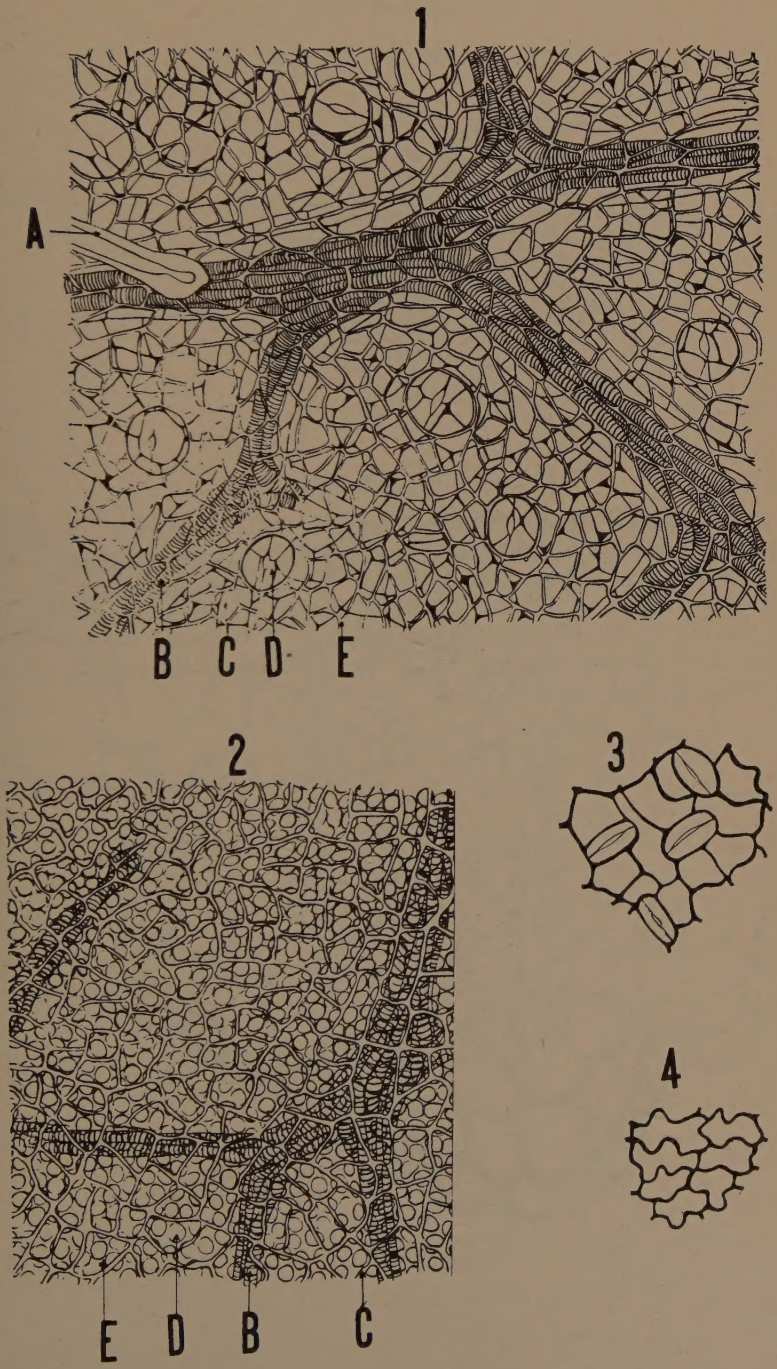


PLATE XXXVI.

1. Cross section of leaf at margin. × 80. A, Cutinized layer of upper epidermis. B, Thick-walled epidermis. C, Palisade cell. D, Spongy parenchyora. E, Cutinized layer of lower epidermis.

2. Cross section of midrib. × 80. A, Cutinized layer of epidermis. B, Epidermis. C, Palisade cells. D, Parenchyma. E, Cutinized layer of lower epidermis. F, Water-conducting tissue. G, Food-conducting tissue. H, Lower epidermis. I, Fiber cells.

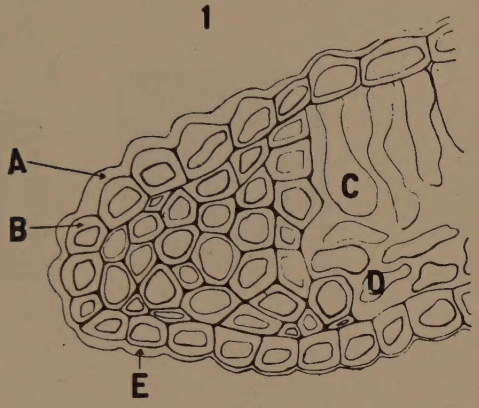
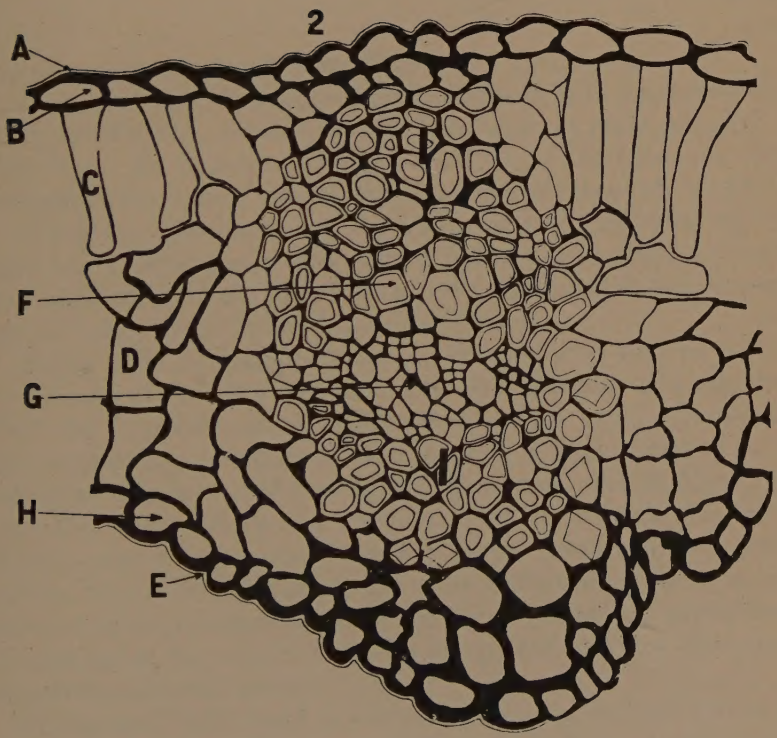



PLATE XXXVI.